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Poietics™ human adipose derived stem cells (ADSC)

Instructions for use

Safety statements

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use *in vitro* diagnostic or clinical procedures.

WARNING: CLONETICS™ AND POIETICS™ PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-I, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th edition. If you require further information, please contact your site safety officer or scientific support.

Unpacking and storage instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells - If there is dry ice left in the package, place cryovials immediately into liquid nitrogen. If no dry ice is left in the package, thaw and use them immediately.
3. BulletKit™ instructions: upon arrival, store basal medium at 2℃ to 8℃ and SingleQuots™ at –20℃ in a freezer that is not self-defrosting. If thawed upon arrival, growth factors can be stored at 4℃ and added to basal medium within 72 hours of receipt. After SingleQuots™ are added to basal medium, use within 1 month. Do not re-freeze.

Preparation of medium

1. Decontaminate the external surfaces of the ADSC-GM SingleQuots™ and the ADSC basal medium (ADSC-BM) bottle with 70% v/v ethanol or isopropanol before placing in the biological safety cabinet (BSC).
2. Make up the complete growth medium by adding the contents of the SingleQuots™ to the basal medium (ADSC-BM).
3. Rinse each SingleQuots™ container with the medium. It may not be possible to recover the entire volume listed for each vial. Small losses should not affect the cell growth characteristics.

Thawing of cells / initiation of culture process

1. The recommended seeding density for adipose derived stem cells is 5,000 cells per cm^2.
2. To set up cultures, calculate the number of vessels needed based on the recommended seeding density and the surface area of the vessels being used. Add the appropriate amount of medium to the vessels (0.2-0.4 ml per cm^2) and allow the vessels to equilibrate in a 37℃, 5% CO_2 humidified incubator for 20 to 30 minutes.
3. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure and then retighten. Quickly thaw the cryovial in a 37℃ water bath.
4. Thaw the vial of cells by swirling it in the waterbath and removing it when the last bit of ice has melted. Do not submerge the vial completely. Thawing the cells for longer then 2 minutes results in less than optimal results.
5. Remove the cryovial immediately, wipe it with 70% alcohol, making sure to remove excess alcohol, and transfer to a sterile field (BSC).
6. Add the calculated volume of cell suspension to each prepared flask and gently rock to disperse the cell suspension over the growth surface.
7. Incubate at 37℃, 5% CO_2 and 90% humidity and allow cells to adhere for several hours (or overnight).
8. When the cells have attached to the growth surface of the flask, replace the medium with an equal volume of fresh, pre-warmed growth medium.

Subculturing

NOTE: Lonza warrants its Clonetics™ cells only if Lonza subculturing reagents are used. The recommended subculturing reagents for these cells are trypsin/EDTA (CC-5012) and trypsin neutralizing solution (CC-5002).

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1. Aseptically remove and discard all of the spent media from the flasks.
2. Wash the attached cell layer with Dulbecco's phosphate buffered saline or an equivalent calcium and magnesium free balanced salt solution. Add the wash solution to the side of the flask opposite the attached cell layer. Rinse by rocking the flask back and forth several times. Aseptically remove and discard the wash solution.
3. Add a sufficient volume of trypsin/EDTA (CC-5012) solution to cover the cell layer (approx. 0.05 ml/cm^2). Gently rock the flask(s) to ensure that the cells are covered by the trypsin solution. Incubate at 37°C for 3 to 5 minutes, and then observe under a microscope. If the cells are less than 90% detached, continue incubating and observe within 2 minutes for complete detachment of the cells. Tapping the flask or plate will expedite cell detachment.
4. When ≥90% of the cells have rounded and detached, stand the flasks on end for a minimal length of time to allow the cells to drain. Add twice the volume used for the trypsin/EDTA of temperature equilibrated TNS (CC-5002) to each vessel. Disperse the solution by pipetting over the cell layer surface several times.
5. Centrifuge cells at approximately 210 x g for 5 minutes at room temperature.
6. Resuspend the cell pellet(s) in a minimal volume of temperature equilibrated ADSC-GM and remove a sample for counting.
7. Count the cells with a hemacytometer or cell counter and calculate the total number of cells and the viability.
8. If necessary, dilute the suspension with ADSC-GM to achieve the desired cell concentration and recount the cells.
9. Assess cell viability using trypan blue.
10. Use the following equation to determine the total number of viable cells.

$$\text{Total \# of viable cells} = \text{Total cell number x percent viability}$$

11. Determine the total number of flasks to inoculate by using the following equation.

$$\text{Total \# of flasks} = \frac{\text{Total \# of viable cells}}{(\text{growth area in cm}^2 \text{ x recommended seeding density in cm}^2)}$$

12. Use the following equation to calculate the volume of cell suspension to seed into your flasks. Determine the volume of ADSC-GM to add to each flask so that the final culture volume is 0.2-0.4 ml per cm^2.

$$\text{Seeding Volume} = \frac{\text{Total volume of diluted cell suspension}}{\text{\# of flasks as determined in step 11}}$$

13. Prepare flasks by labeling each flask with the passage number, strain number, cell type and date.
14. Add the appropriate volume of temperature equilibrated ADSC-GM as determined in step 12.
15. Incubate at 37°C, 5% CO_2 and 90% humidity.
16. 3 to 4 days after seeding, completely remove the medium. Replace with an equal volume of ADSC-GM.

Maintenance

1. ADSC cultures should be fed every 3 to 4 days after plating until the cells are subcultured.
2. To feed the cultures, gently and completely remove the ADSC-GM from the culture vessel.
3. Replace with an equal volume of temperature equilibrated ADSC-GM and return the culture vessels to the incubator.
4. When seeded at 5,000 cells per cm^2 of surface area, ADSC should be near confluence by day 6 or 7. The ADSC should be subcultured when they are just sub-confluent (approximately 90% confluent).

Ordering information

PT-5006	ADSC, adipose-derived stem cells, cryopreserved	≥ 1,000,000 cells
PT-5007	ADSC, adipose-derived stem cells, cryopreserved (Diabetes Type I)	≥ 1,000,000 cells
PT-5008	ADSC, adipose-derived stem cells, cryopreserved (Diabetes Type II)	≥ 1,000,000 cells
PT-3273	ADSC-BM, adipose derived stem cell basal medium	500 ml
PT-4503	ADSC-GM SingleQuots™, formulates ADSC-BM to ADSC-GM	
CC-5012	Trypsin/EDTA	100 ml
CC-5002	Trypsin neutralizing solution	100 ml

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Product warranty

CULTURES HAVE A FINITE LIFESPAN *IN VITRO*. Lonza warrants its cells only if Poietics™ media are used, and the recommended protocols are followed. Cryopreserved ADSC are assured to be viable and functional when thawed and maintained properly.

Quality control

All cells are performance assayed and test negative for HIV-1, hepatitis-B & C, mycoplasma, bacteria, yeast and fungi. Cell viability and morphology are measured after recovery from cryopreservation. Non-routine performance and quality testing to meet your specifications is available for an additional fee.

For detailed information concerning QC testing, please refer to the certificate of analysis.

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